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J. Insect Physiol., 1969, *Vol.* 15, *pp.* 893 *to* 898. *Pergamon Press. Printed in Great Britain*

THE ALARM-DEFENCE SYSTEM OF THE ANT *LASIUS ALIENUS*

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(*Received* 21 *October* 1968)

Abstract—The principal volatile products of the formicine ant *Lasius alienus* were identified, their glandular sources located in part, and their functions analysed. The more abundant substances in the C_9–C_{10} range were shown to be alarm substances, with behavioral threshold concentrations estimated to vary in order of magnitude from 10^7 to 10^{11} molecules/cm^3. Undecane, the principal component, was shown to be more effective as an alarm substance for *L. alienus*, whose estimated response threshold is 10^7 to 10^{10} molecules/cm^3, than for the related species *Acanthomyops claviger*, whose estimated threshold is 10^{10} to 10^{12} molecules/cm^3. *L. alienus* appears to depend less in its defence strategy on orientation toward points of disturbances and defensive chemical sprays, and more on 'early warning' and evacuation, than does *A. claviger*.

INTRODUCTION

THIS article is one of a series on chemical defence and pheromone-mediated alarm behaviour in the ant subfamily Formicinae. Our goal has been to examine in detail the chemistry and function of volatile secretions in several formicine species, to reconstruct the evolution of the chemical systems at the species level, and to elucidate the relations of the communications and defensive functions of the volatile secretory substances. In an earlier article (REGNIER and WILSON, 1968) we presented the results of a study of *Acanthomyops claviger*, a representative of a peculiar Nearctic genus all of whose species are pale coloured (in the worker caste), subterranean, and temporarily parasitic in their mode of colony founding (WING, 1968). In this article we will describe the chemical alarm-defence of *Lasius alienus*, a typical member of the genus generally considered by taxonomists to have given rise to *Acanthomyops*. *L. alienus* is non-parasitic and is found throughout the north temperate zone, although the Eurasian and North American populations are slightly different and may represent distinct species (WILSON, 1955). Its workers, unlike those of *A. claviger*, commonly forage above ground and are darkly pigmented and large-eyed. Thus, although the two species are phylogenetically rather close, they differ strongly in several key aspects of their ecology.

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PROCEDURES IN CHEMICAL ANALYSIS

Source of materials

Citronellol, citronellal, 2,6-dimethyl-5-hepten-1-al, and n-undecane were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, Tridecane and 2-tridecanone were purchased from Eastman Organic Chemicals, Rochester, New York. K and K Laboratories, Plainview, New York, were the suppliers of 2-pentadecanone. Precoated gas chromatography packing materials were obtained from Applied Science Laboratories, State College, Pennsylvania. Colonies of *L. alienus* were collected in the Boston, Massachusetts, area and maintained in culture in the laboratory.

Gas chromatographic analyses

Gas chromatographic analyses were carried out on an F & M 402 gas chromatograph equipped with hydrogen flame detectors and a 6 ft × 0·25 in. glass column packed with 3% OV-17 on 100-120 mesh Gas Chrom Q. The detector and inlet temperatures were 200 and 100°C respectively while the helium flow rate was 30 ml/min. Peak areas were measured by cutting the peaks out of the chromatogram and weighing them. Quantitation of volatile components was achieved by the internal standard technique described previously by REGNIER and WILSON (1968).

Combination mass spectrometric–gas chromatographic (MS–GC) analyses

The mass spectrometer was operated at an electron energy of 70 eV with ion source and molecular separator temperatures of 250 and 150°C respectively. The gas chromatograph was fitted with an 8 ft × 0·25 in. glass column packed with 3% OV-17 on Gas Chrom Q. Gas chromatographic analyses were carried out on the combination instrument by temperature programming from 100 to 250°C.

Extraction of exocrine gland substituents

Crude extracts for gas chromatographic analysis were prepared by crushing the bodies or glands of *L. alienus* workers in carbon disulphide and extracting for 12 hr at room temperature.

RESULTS OF CHEMICAL ANALYSIS

Exocrine gland secretions of L. alienus *workers*

Volatile organic compounds were extracted from crushed tissue with carbon disulphide resulting in a total tissue extract. Carbon disulphide was used as a solvent because of its low response in the hydrogen flame detector of the gas chromatograph. Using low-response solvents, it is possible to analyse very small quantities of volatile odorants gas-chromatographically without having the solvent mask the odorant. Gas chromatographic separation of the total tissue extract on an OV-17 column showed that the extract contained at least twelve volatile components (Fig. 1). Identification of these compounds was achieved by comparing the mass spectral–gas chromatographic (MS–GC) properties of an unknown with standard compounds. An identification was considered positive if the MS–GC properties of an unknown and standard compound were identical. Since all of the compounds identified in these extracts have been reported in other, closely related species of *Lasius* and *Acanthomyops*, no mass spectral data will be presented here.

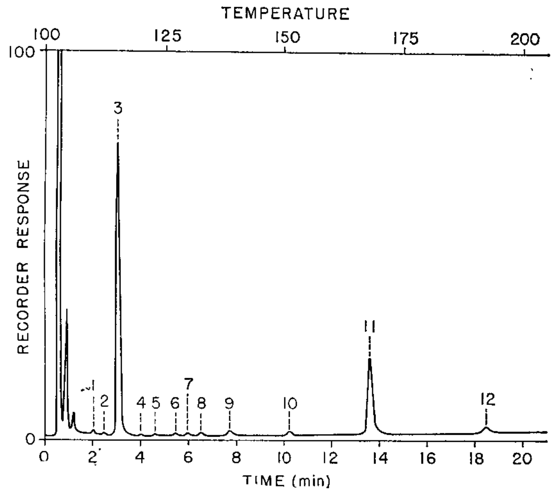

FIG. 1. Gas chromatogram of single worker of *L. alienus*.

TABLE 1—CONSTITUENTS OF THE EXOCRINE GLAND SECRETIONS OBTAINED FROM *L. alienus* WORKERS

GC No.*	Molecular ion†	Compound	ng/worker‡ (mean)
1	—	Unknown	Trace
2	—	Unknown	Trace
3	156	Undecane	1400
4	—	2,6-Dimethyl-5-hepten-1-al §	2
5	—	Unknown	3
6	—	2,6-Dimethyl-5-hepten-1-ol §	7
7	154	Citronellal	8
8	184	Tridecane	11
9	156	Citronellol	16
10	—	Unknown	7
11	198	2-Tridecanone	770
12	226	2-Pentadecanone	37

* The gas chromatographic fraction numbers indicated are those in the chromatogram of Fig. 1.

† Apparent molecular ions were obtained by mass spectrometry.

‡ Compounds 4- to 10 vary as much as ±50 per cent relative to the concentrations of undecane and 2-tridecanone.

§ These identifications are based on gas chromatographic retention times alone (see text).

Excised Dufour's glands were found to contain compounds 3, 5, 8, 11, and 12 of Fig. 1 by gas chromatographic analysis of glandular extracts. These compounds have been identified as n-undecane, n-tridecane, 2-tridecanone, and 2-pentadecanone as in the quantities given in Table 1. The small quantity of component 5 did not permit identification of this compound. The principal constituents of Dufour's gland are n-undecane and 2-tridecanone. These compounds were found to be present in concentrations of 1·4 and 0·77 μg respectively/gland. The total concentration of organic compounds in Dufour's gland constitutes 0·17 per cent of the body weight of the workers.

Compounds 7 and 9 of Fig. 1 have been identified as citronellal and citronellol by combination MS–GC analysis. Compounds 4 and 6 are probably 2,6-dimethyl-5-hepten-1-al and 2,6-dimethyl-5-hepten-1-ol on the basis of gas chromatographic retention times. These substances have been identified in related ant species on the basis of mass spectral features (LAW *et al.*, 1965). The low concentrations of compounds 1, 2, and 10 in the mixture prevented their MS–GC characterization. The terpenes in the extracts are probably associated with the mandibular gland as is the case of closely related species such as *L. umbratus* and *A. claviger*. Relative

TABLE 2—DATA FROM BEHAVIORAL EXPERIMENTS AND ESTIMATED K OF *L. alienus* SECRETORY PRODUCTS

Substance	Nest temp. (°C)	D: Diffusion coefficient	Q: Evaporation from artificial source (mol/sec)	r: Distance to ant (cm)	t: Time to response (sec)	Estimated K threshold concentration (mol/cm^3)
Formic acid	27	0·18	$> 10^{15}$	$\geqslant 2$	—	No responses in 35 sec
Undecane	27	0·06	$5{\cdot}02 \times 10^{12}$	2·2	2	$4{\cdot}36 \times 10^{7}$
	27	0·06	$5{\cdot}02 \times 10^{12}$	1·8	2	$1{\cdot}78 \times 10^{9}$
	27	0·06	$5{\cdot}02 \times 10^{12}$	1·0	1	$1{\cdot}67 \times 10^{10}$
	27	0·06	$5{\cdot}02 \times 10^{12}$	1·6	3	$6{\cdot}39 \times 10^{10}$
	27	0·06	$5{\cdot}02 \times 10^{12}$	1·6	2	$9{\cdot}13 \times 10^{9}$
	27	0·06	$5{\cdot}02 \times 10^{12}$	6·5	64	$3{\cdot}90 \times 10^{10}$
Citronellal	27	0·06	$5{\cdot}65 \times 10^{11}$	2·5	16	$4{\cdot}27 \times 10^{10}$
	27	0·06	$5{\cdot}65 \times 10^{11}$	1·0	2·5	$1{\cdot}02 \times 10^{11}$
	27	0·06	$5{\cdot}65 \times 10^{11}$	1·0	2·5	$1{\cdot}02 \times 10^{11}$
	27	0·06	$5{\cdot}65 \times 10^{11}$	2·0	25	$1{\cdot}86 \times 10^{11}$
Citronellol	27	0·06	$4{\cdot}85 \times 10^{11}$	1·4	4	$3{\cdot}98 \times 10^{10}$
	27	0·06	$4{\cdot}85 \times 10^{11}$	2·5	3	$1{\cdot}61 \times 10^{7}$
	27	0·06	$4{\cdot}85 \times 10^{11}$	3·3	38	$4{\cdot}77 \times 10^{10}$
2,6-Dimethyl-5-hepten-1-ol	27	0·06	$8{\cdot}46 \times 10^{12}$	1·5	5	$7{\cdot}90 \times 10^{11}$
	27	0·06	$8{\cdot}46 \times 10^{12}$	1·8	7	$6{\cdot}18 \times 10^{11}$
	27	0·06	$8{\cdot}46 \times 10^{12}$	2·0	7	$3{\cdot}27 \times 10^{11}$
	27	0·06	$8{\cdot}46 \times 10^{12}$	1·5	3·5	$3{\cdot}09 \times 10^{11}$

Except for formic acid, each row represents a single response.

concentrations of these compounds were found to vary as much as 50 per cent in the total extracts.

ANALYSIS OF FUNCTIONS

The mandibular gland and Dufour's gland secretions of the *L. alienus*, like those in *A. claviger*, have at least two functions. When sprayed from the poison nozzle of the abdomen onto the body of an enemy, undecane serves as a spreading agent for formic acid (REGNIER and WILSON, 1968). Also, undecane and the volatile compounds of lower molecular weight, with the exception of formic acid, serve as alarm substances. We found alarm response of *L. alienus* to be more frenetic than that of *A. claviger*. The workers are easily triggered into excited and prolonged running. Orientation is frequently toward the odour source, but at any given moment the majority of alarmed workers appear to be moving in an erratic, aimless fashion through the nest. Thus, whereas *A. claviger* workers tend (with many exceptions) to travel in a more or less deliberate fashion toward the odour source, *L. alienus* workers tend to scatter away from it. Threshold concentrations of the *L. alienus* volatiles were estimated by the diffusion model method of WILSON *et al.* (1969) and are presented in Table 2.

DISCUSSION

Alarm communication in *L. alienus* differs from that in *A. claviger* in two seemingly related aspects. First, alarmed *alienus* workers run more rapidly and orient less to the odour source. Consequently they disperse more rapidly from serious disturbances. Second, *L. alienus* workers have much lower response thresholds to undecane, one of the principal volatile components in both species. They react to undecane at concentrations of 10^7 to 10^{10} molecules/cm^3, whereas *A. claviger* workers react at 10^{10} to 10^{12}. The alarm signal therefore travels faster in *L. alienus*, and the fast portion of the signal extends farther away from the signalling ant.

We conclude that the two species display examples of different alarm-defence strategies. *A. claviger* forms very large colonies which, at certain times of the summer at least, are densely concentrated in earthen galleries. They are slower to alarm and tend to move more in the direction of the disturbance. They also have better combat equipment, in the form of large quantities of citronellal and citral stored in hypertrophied mandibular gland reservoirs. *L. alienus*, on the other hand, forms smaller, usually more dispersed colonies. In the North American population nests are typically constructed in rotting logs and stumps that provide many more escape routes than do the earthen nests of *Acanthomyops*. It therefore seems consistent that *L. alienus* employs a more rapid alarm signal and depends more on evacuation than does *A. claviger*.

Acknowledgements—We wish to thank Dr. CHARLES C. SWEELY for placing the combination gas chromatograph–mass spectrometer at our disposal, and Dr. BARRY P. MOORE and Mr. JACK M. REICHSON for help and advice given during the study. The project was supported by National Science Foundation Grants GB-5079 and GB-5706.

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